Genome Engineering and Biosensors

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Outline

Applications

- Biofabrication
- Biomaterials
- Bioproduction of industrial chemicals

Basic Enabling Technologies

- Genome Engineering
- Biosensors
- Orthogonally interacting protein pairs
- DNA synthesis

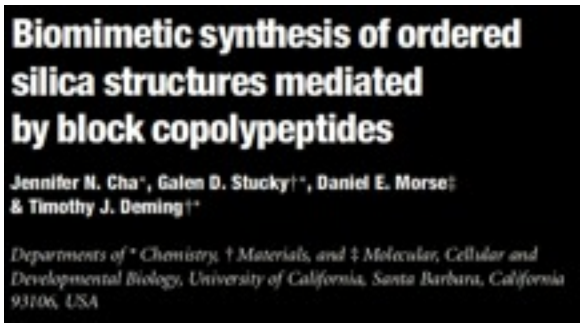
Biofabrication
- silicatein alpha

Overview | why biofabricate machines and materials?

Goal | to make functional components of mechanical, electrical, optical and fluidic systems.

Approach

1. Clone biological pathways for manipulation of metals, silicon, gels and other substrates.
2. Couple to a sensing element of choice.
3. Integrate into proper support.


Biomimetic synthesis of ordered silica structures mediated by block copolypeptides

Jennifer N. Cha*, Galen D. Stucky†*, Daniel E. Morse‡ & Timothy J. Deming†*

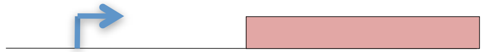
*Departments of * Chemistry, † Materials, and ‡ Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, California 93106, USA*

Cha et al, Nature 403, 289-292 (2000)

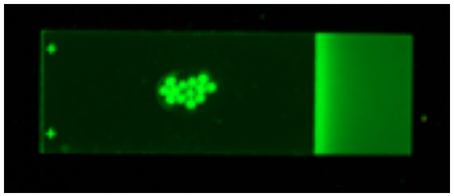
Biofabrication of electro-optic component from light

Blue light sensor

Silicatein A

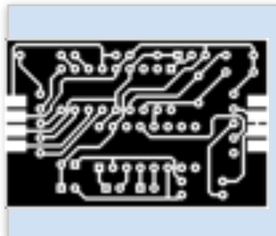


Embedding of bacteria in support (PDMS)

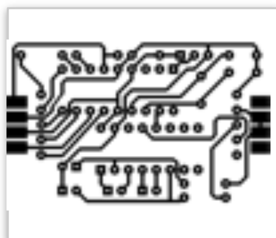


Alginate-silica beads as an easy route for bacterial embedding in PDMS and other mechanical supports (plastics, alloys)

Support doped with metal/silicon salt and with embedded bacteria



Illuminating with blue light through a photomask



Patterned expression of Silicatein A & synthesis of metal conductors

Biomaterials
- silk

Engineering silk

Properties of silk

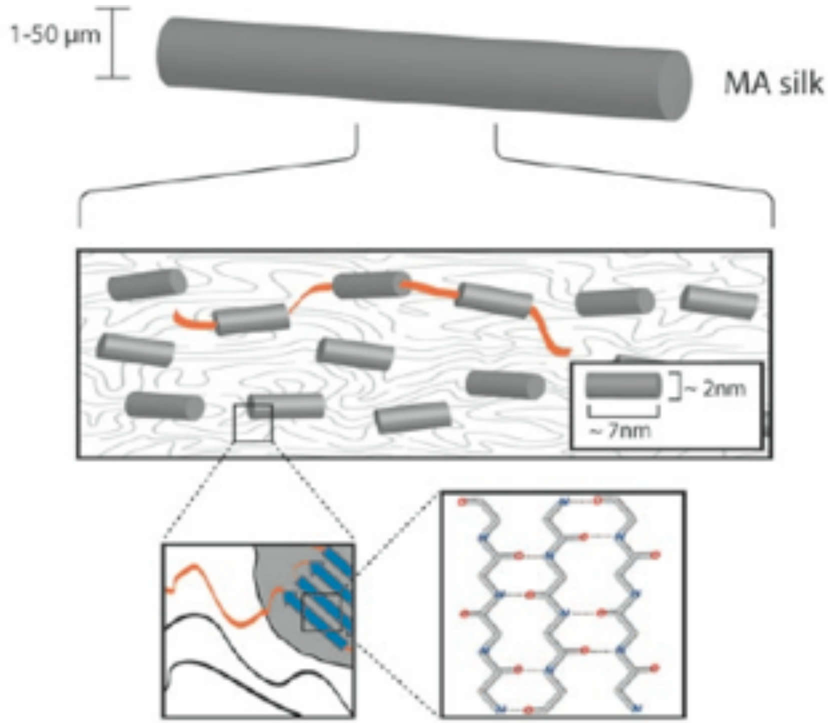
* silk is one of the strongest natural fibers
* tensile strength comparable to high-grade steel
* extremely lightweight
* highly ductile

Applications

* suture material (stitching)
* wound healing bandages
* tendon/ligament repair
* bulletproof vests
* lightweight structural fabrics
* athletic clothing

Chemical composition of spider silk

* comprises of proteins with many repeat sequences
* rich in Ala and Gly enabling tight pacing
* interspersed crystalline and amorphous regions



OBJECTIVES

Producing silk with desired properties

Expressing spider silk in silkworms ?

Well-established rearing and extraction protocols for silkworms

Need for tools to genetically modifying silkworms

- Transgenesis efficiency: 1 transgenic worm out of 100 injected eggs
- Need homologous recombination to completely engineer silk properties
- Developing selection marker – diptheria toxin ?

Production of Genetically-modified Silk

Bioproduction

- lycopene
- tryptophan

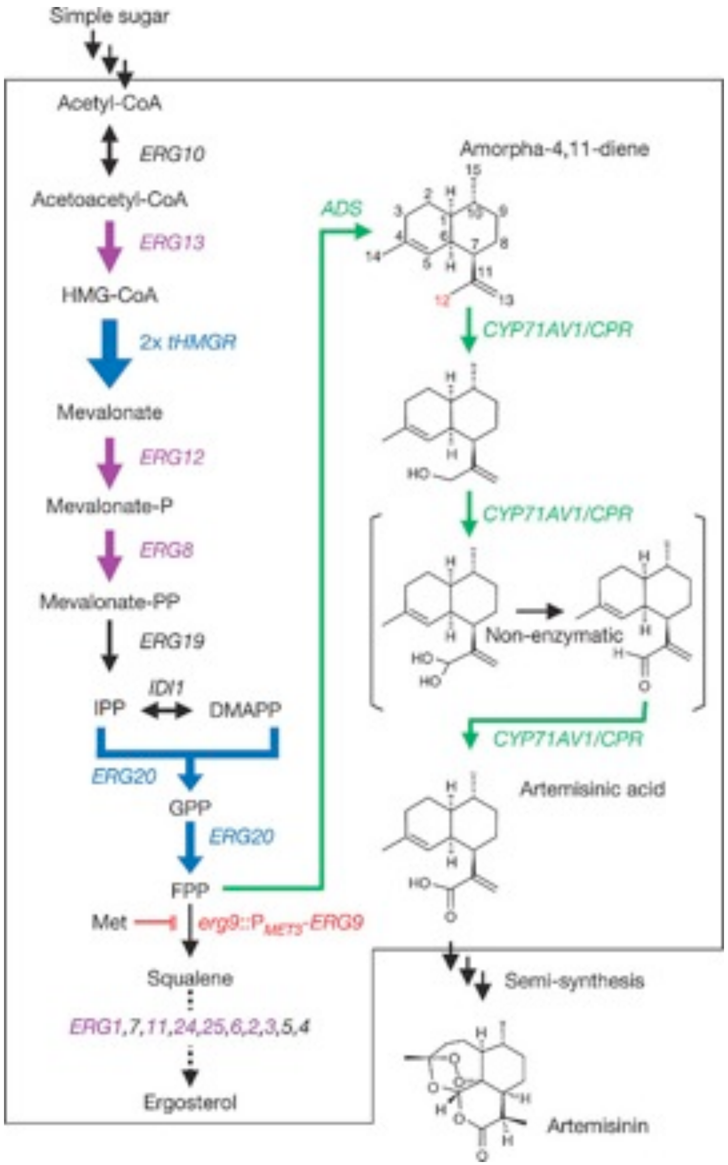
Bioproduction of chemicals

* Using microorganisms for mass production of chemicals
* Modification of existing biosynthetic/metabolic pathways
* High yields compared to total chemical synthesis or isolation
* Enzymes can make exquisite stereospecific compounds

Challenges

* Identification of the "gene cluster"
* Found in organisms without genetic engineering tools developed
* Heterologous expression in tractable host like E coli

An early success of synthetic biology



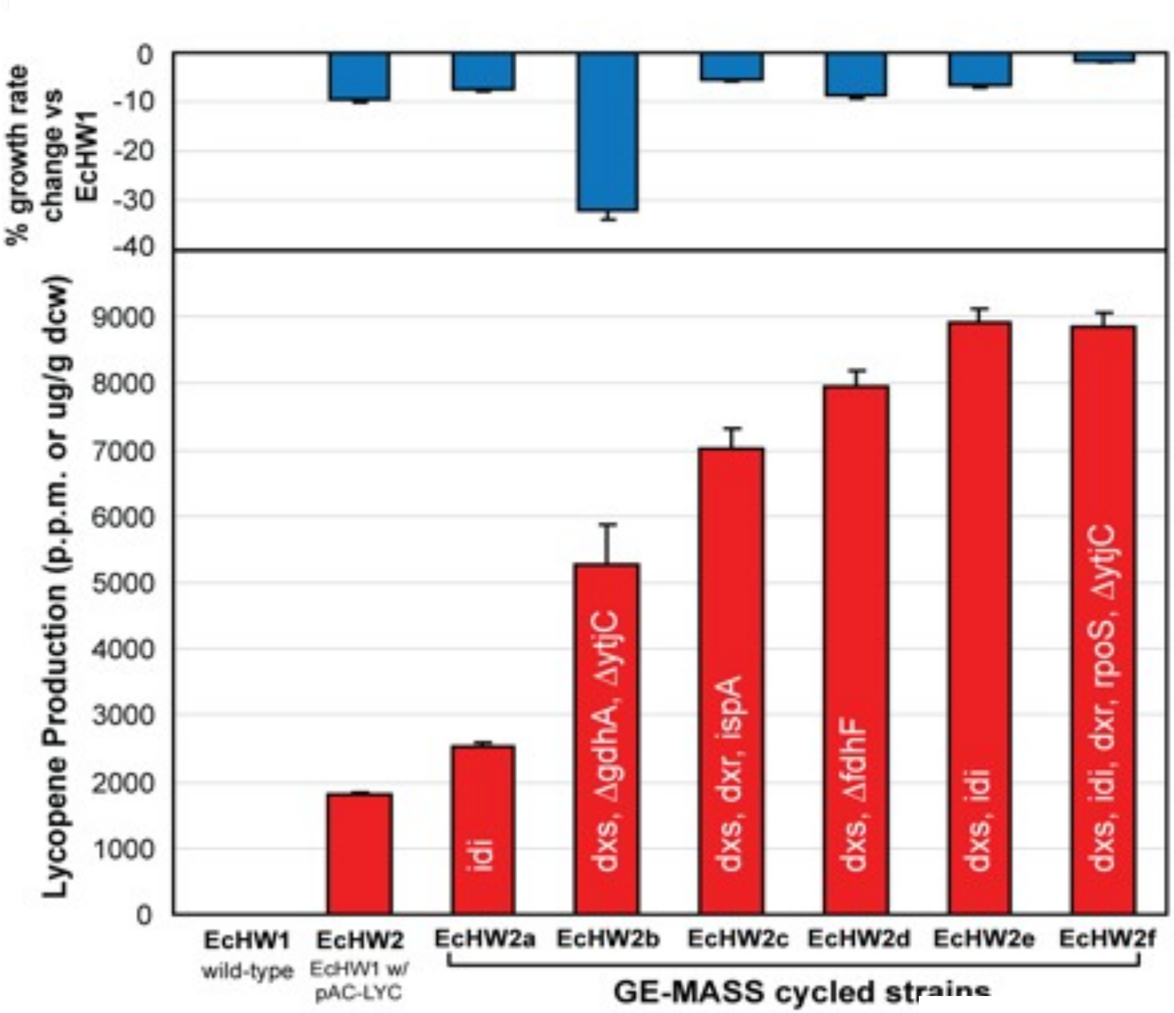
Production of artemisinic acid – precursor of anti malarial compound

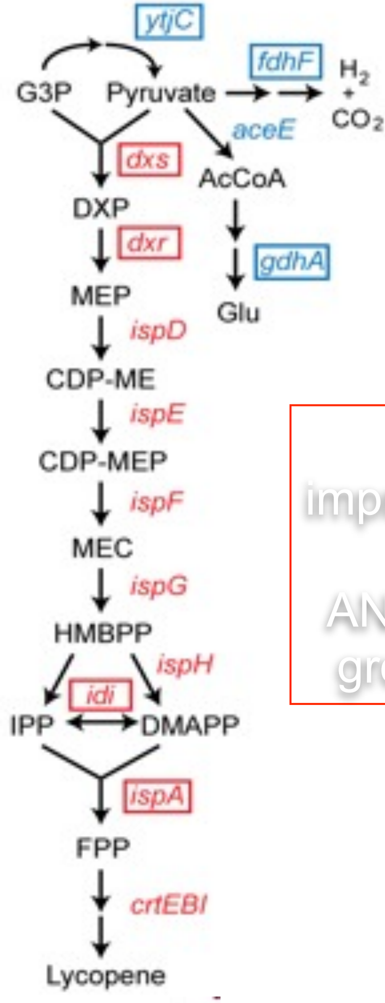
Heterologous expression of a plant enzyme ADS and 8-gene mevalonate pathway from S.cerevisiae

Ro, Paradise and Keasling, Nature, 2006

Accelerated Evolution 23K combinations per gene

Lycopene (hydrocarbon): 20 genes up, 4 down, 2 new

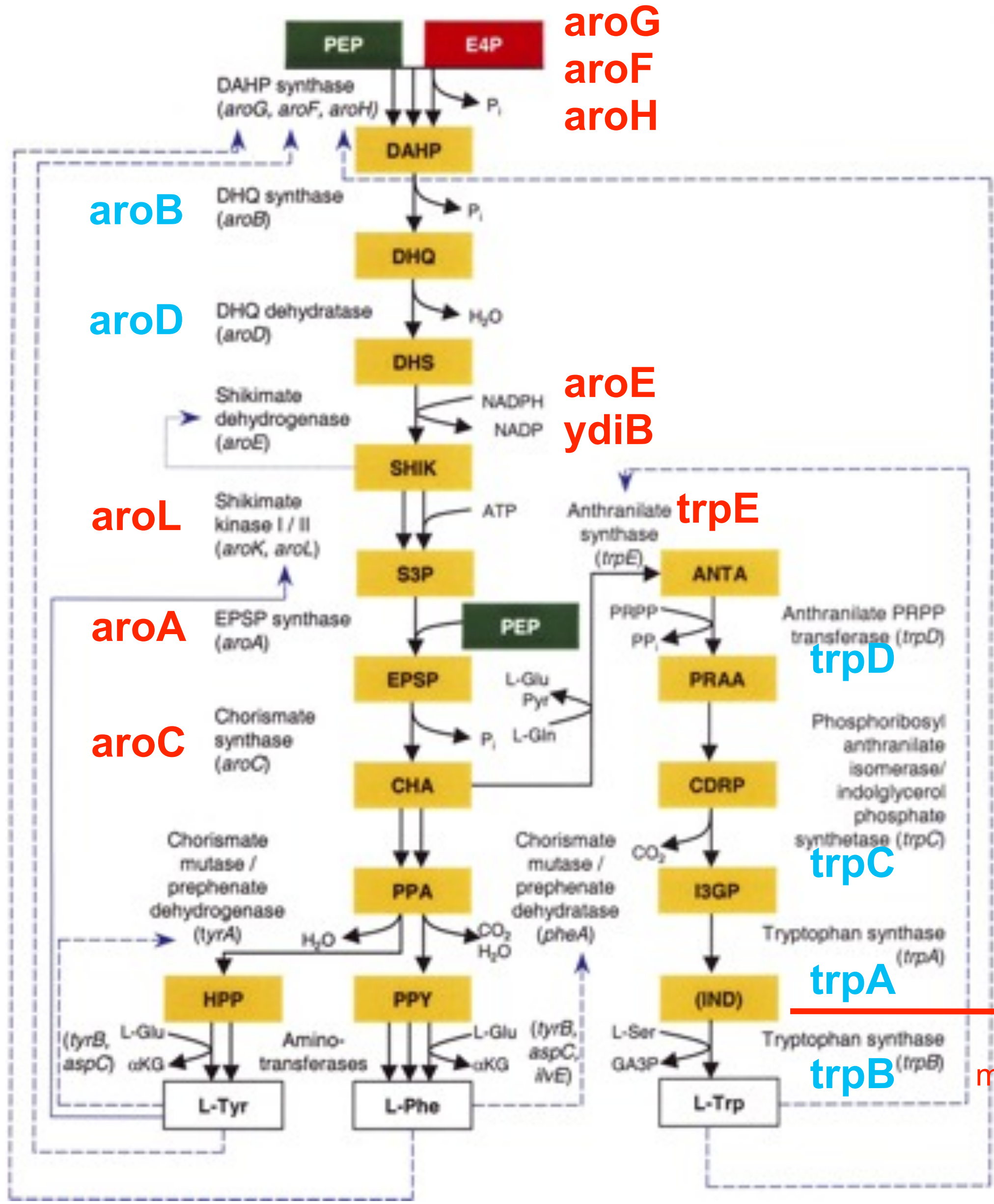




5-fold improvement in 3 days
AND improve growth rates

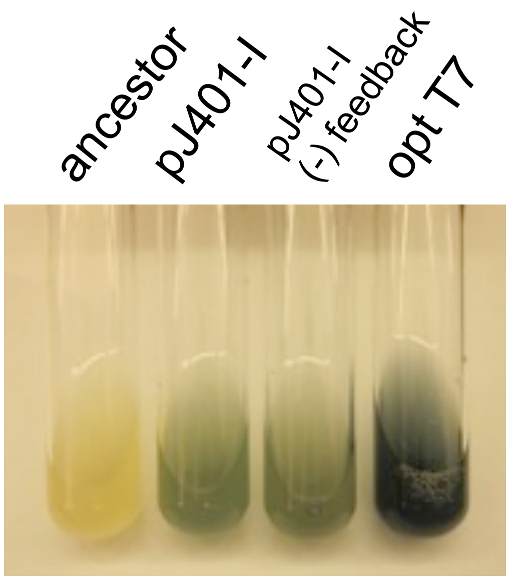
Wang H et al Nature 2009

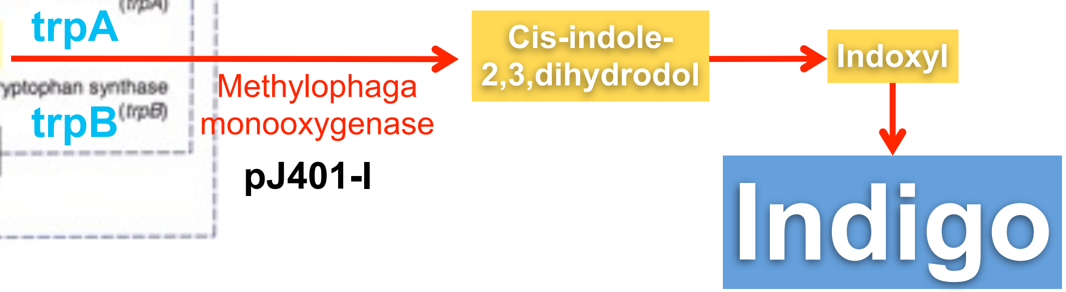
Native Pathway Engineering with Synthetic Promoters



Bongaerts, Metabol Eng 2001

Insertion of T7 promoter into 12 different operons for and T7 RNAP regulated gene expression





Outline

Applications

- Biomaterials

  Biofabrication of circuits

  Engineered silk
- Biofilms – microbial consortium
- Production of industrial chemicals

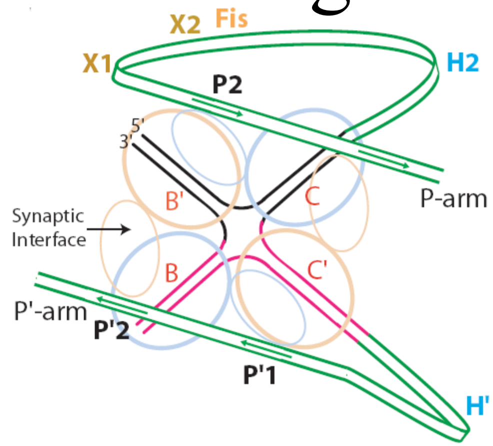
  Lycopene

  Tryptophan

Basic Enabling Technologies

- Genome Engineering
- Biosensors
- Orthogonally interacting protein pairs
- DNA synthesis

3 Protein-directed recombination strategies



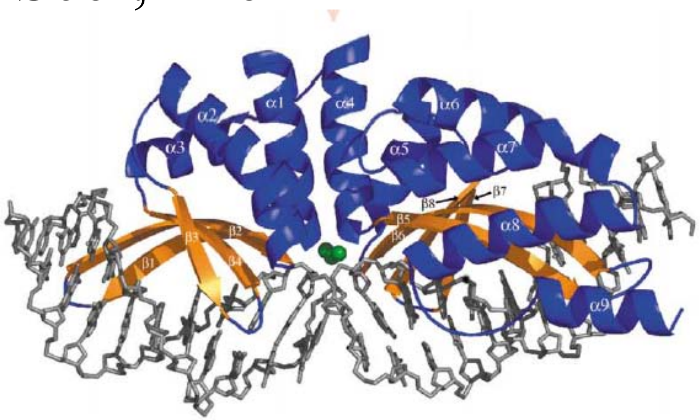
1. Integrase/recombinase

λ(Gateway)

ϕC31, Cre-lox

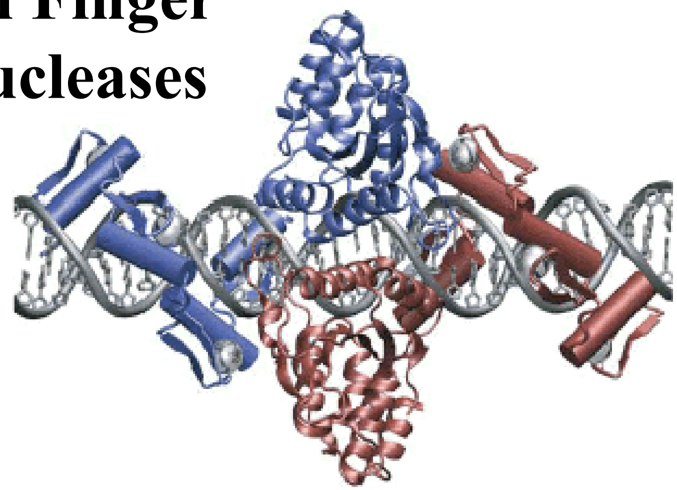
2. Meganucleases

SceI, DreI



E-*DreI* (Engineered I-DmoI / I-CreI)

3. Zn Finger Nucleases

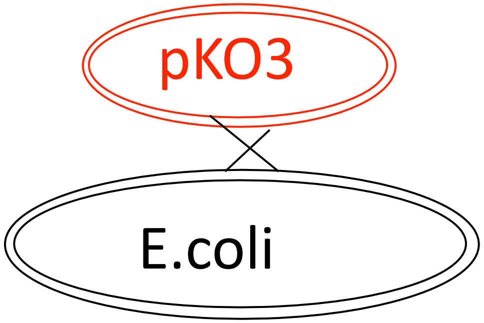


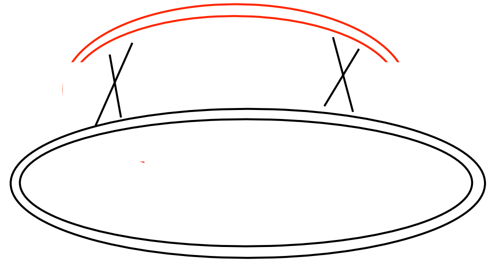
• Design, activity, and structure of a highly specific artificial endonuclease. Mol Cell. 2002 Chevalier, Kortemme, Chadsey, Baker, Monnat, Stoddard.

• A conformational switch controls the DNA cleavage activity of lambda integrase. Mol Cell. 2003 Aihara, Kwon, Nunes-Duby, Landy, Ellenberger

29

Support 4 Genome engineering strategies

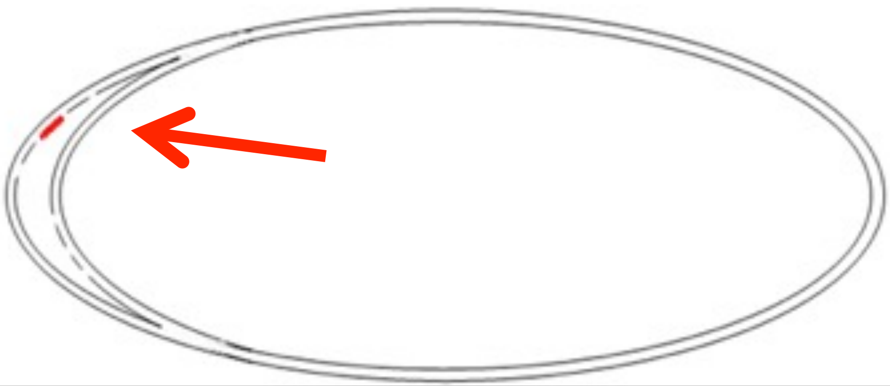




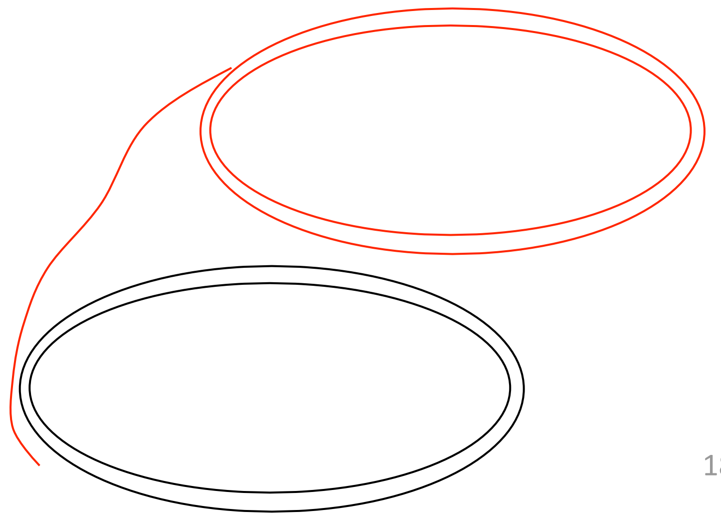
#1: ds-Circle x Circle
2 step recA+ recombination
Select + counterselect
Link et al J. Bact 1997
(Open-access)

#2: ds-Linear x Circle
1 step 5’>3’exo Reda/E b/T
Select
Zhang et al Nat.Gen 1998 Yu et al. PNAS 2000
(GeneBridges license)

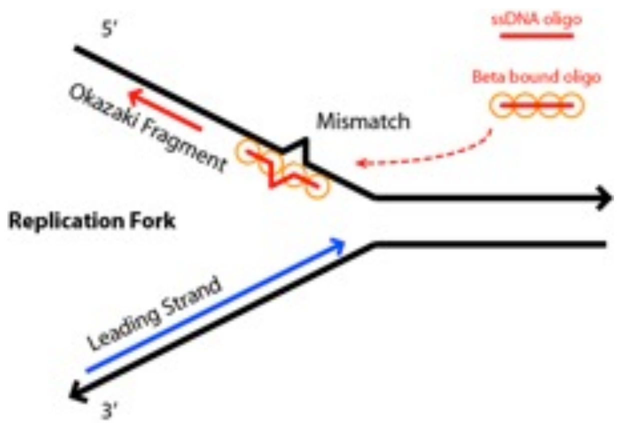
#3: ss-90mer x ds-Circle
Costantino &Court PNAS’03



#4: ss-Mb x ds-Circle conjugation

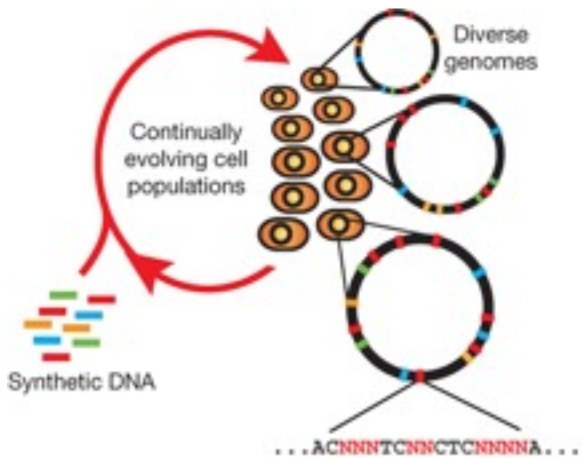


Multiplex Automated Genome Engineering (MAGE)



Allelic Replacement

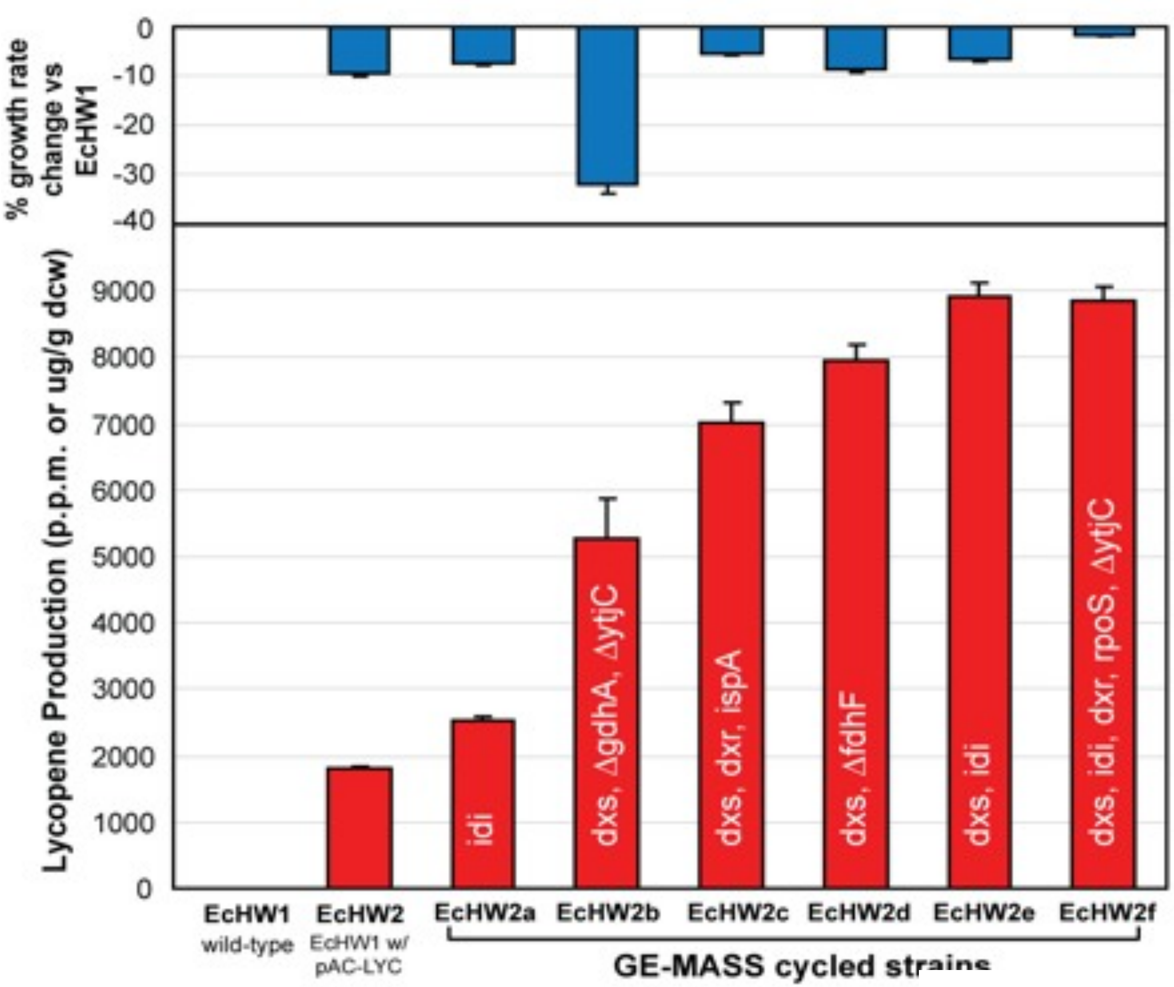
- Strain: MG1655, ΔmutS, integrated λ-Red
- Highly complex oligo pools for multiplexed multi-loci modifications
- >4 billion bp of targeted genetic variation produced per day

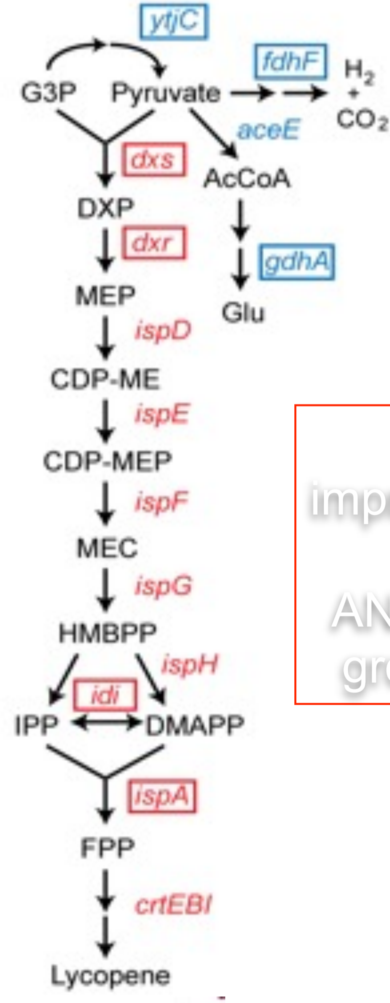


Tool for accelerating evolution of an organism in the lab

Accelerated Evolution 23K combinations per gene

Lycopene (hydrocarbon): 20 genes up, 4 down, 2 new





5-fold improvement in 3 days AND improve growth rates

Wang H et al Nature 2009

Small-molecule biosensors invivo

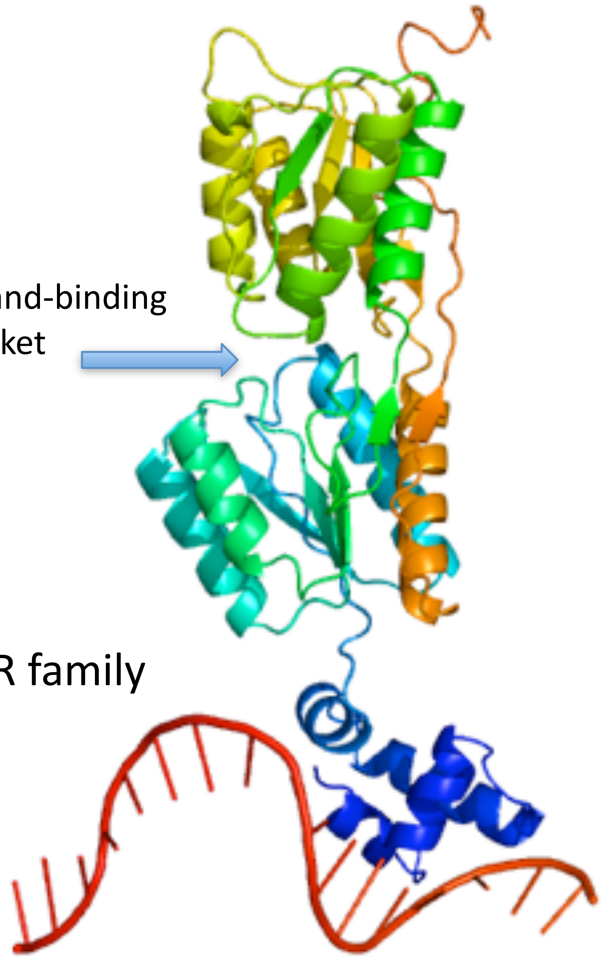
- Transcriptional control : protein-DNA-ligand
- Translation control : Riboswitches-mRNA-ligand
- Post-translational control : protein-protein-ligand

Family of bacterial transcription

Transcriptional control

DNA binding proteins: ada araC arcA argPR carP cpxR crp cspA cynR cysB cytR deoR dnaA dgsA fadR farR fhlA flhCD fnr fruR fur galR gcvA glpR hipB iclR ilvY lacI lexA lrp malT marR melR metJ metR modE nagC narL narP ntrC ompR oxyR pdhR phoB purR rhaS rpoE rpoH rpoN rpoS soxS torR trpR tyrR

Ligand-binding pocket



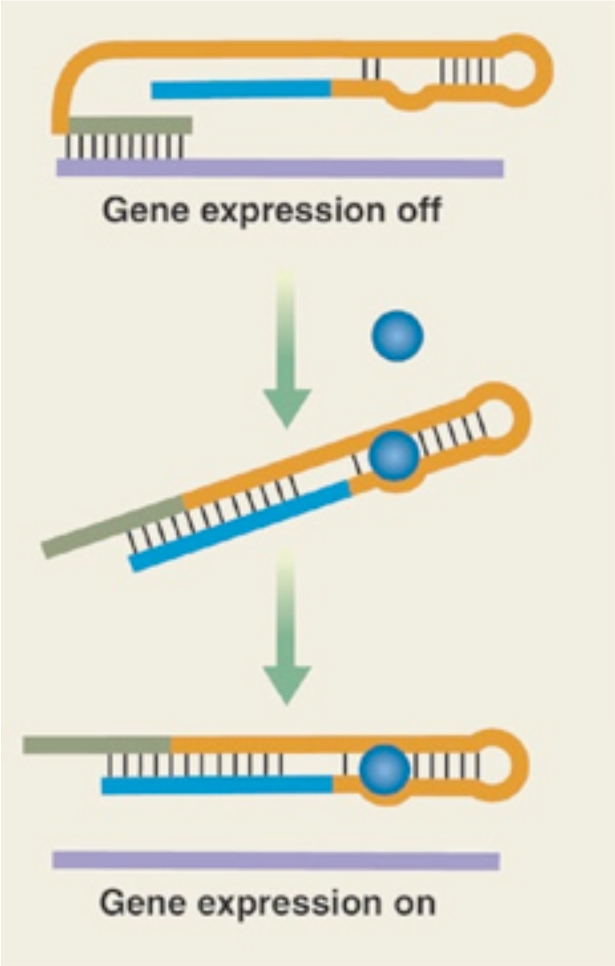
LacI/GalR family

- Ligand-activated
- Allosteric (built-in switch)
- Specificity switch for new ligands
- Potentially tight transcriptional control

Riboswitch - attenuators

Translational control

Adenine B12 FMN Guanine
Glucosamine-6-phosphate Glycine di-GMP
Lysine Molybdenum PreQ1 SAM SAH TPP
theophylline 3-methylxanthine



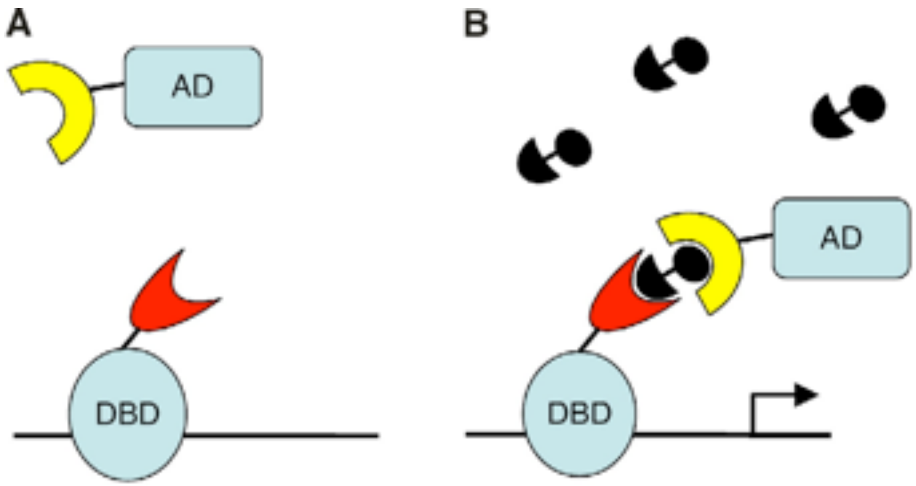
- Ligand-activated
- Allosteric (built-in switch)
- Sequence-specific: anti-sense binds mRNA
- Specificity change can be designed

Breaker, Science, 2008
Bayer and Smolke, Nature Biotech, 2005
Isaacs et al, Nature Biotech, 2004

Ligand-dependent two-hybrid style

Post-translational control

- Protein domains interact when small-molecule is present. Eg: rapamycin
- DBD – ZFP, AD – NFkB domain
- AD recruits RNApol
- DNA and ligand specificity change can be designed
- Other reporters :
    - screen – split GFP, split luciferase
    - selection – split DHFR



Ho, Biggar, Spencer, Schreiber and Crabtree, Nature, 1996

Acknowledgments

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Biofabrication:
Ido Bachelet
Tara Gianoulis

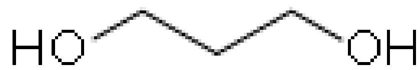
Biomaterials – Silk:
Feng Zhang
Sri Kosuri

Bioproduction – Lycopene and Trp
Harris Wang
Farren Isaacs

Rest of the Church lab

Genome engineering 2000-2006

Dupont: 1,3 Propanediol
(7 years & **$400M** R&D)
135 g/l at 3.5 g/l/h
90% of theoretical yield from glucose



for Sorona polymers

27 changes to 4.6 Mbp *E.coli*
6 genes up, 13 down, 8 foreign genes

Yeast — **DAR1** → **Glycerol-3-P**, **GPP2** → **Glycerol** (**- NADH**)

Klebsiella — ***dhaB1-3*** → **3HPA** (**coB12**)

E.coli — ***yqhD*** → **1,3 propanediol** (**- NADPH**)